Optimization of Live Cell Imaging on Titanium Surfaces

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ABSTRACT

Titanium is a viable material for prosthetic implants due to its biocompatible surface which facilitates cell adhesion. In this study, titanium discs are used as a substrate for bone marrow stromal cells from a rodent. The stromal cells have been genetically altered with Green Fluorescent Protein (GFP) in order to utilize fluorescent microscopy for cell growth analysis.

Previous research by a graduate student focused on automatically recording periodic images of live cell growth using image acquisition software coupled with a fluorescent microscope camera (Jensen 2013). During the duration of these trials, the cells were enclosed in a micro-incubator system which replicated the environment of the control incubator. The micro-incubator was set to maintain an internal temperature of 37.0°C and a 5% CO_2 / 95% air atmosphere. This research focused on investigating different ways to optimize the environment of the micro-incubator, since it was concluded from Jensen's work that there was a huge disparity between the cells' life cycle, adhesion, and expansion in the control incubator environment versus that of the micro-incubator environment.

MATERIALS AND METHODS

Temperature Experiment

For the temperature experiments, the micro-incubator was filled with 12 mL of α-MEM Growth Media + 10% by volume FBS. It was placed on the fluoroscope stage and prepared as though a live cell imaging experiment was to be performed, as detailed in Jensen. The changes in this procedure included greasing the thermo-resister with Dow Corning 705 silicon diffusion pump oil and inserting it into the side of the micron-incubator to measure the micro-incubator's external temperature. The thermocouple, used to measure the micro-incubator's internal temperature was then placed inside the Tygon tubing lead until it hit the bottom of the Teflon well inside the micro-incubator. The set temperature on the controller was then set at 37.0°C, the optimal temperature for rat cell growth. Once the external temperature read by the thermo-resistor reached 37.0°C, the timer was started and the temperatures were recorded for a total of 30 minutes.

Poison Experiment

The three "poisons" encountered by the cell suspension in the micro-incubator that were not present in the control incubator were 1) 316 surgical steel, 2) Dow Corning 734 silicon sealant, and 3) the steel used to make the top of the micro-incubator. The locations of these poisons are highlighted in Figure 1. The cells were thawed, fed, and passaged, following standard cellular biology techniques. After passaging, the appropriate amount of cell suspension needed for the desired viable inoculation density and its balance of growth media was placed on the titanium discs that were within silicon wells. These wells were placed in six wells plates. For each poison trial, two control wells and two poisoned wells were used. The six well plates were placed in the control incubator and the time was recorded. Fluoroscope pictures were then taken every 24 hours for one week using the Image-Pro Plus digital and video capture software in conjunction with an Olympus DP72 camera and an Olympus BX41 fluorescent microscope.

REFERENCES

1. Jensen, Rebecca Leah. Live Cell Imaging to Investigate Bone Marrow Stromal Cell Adhesion and Migration on Titanium Surfaces: A Micro-Incubator *In Vitro* Model. Master's Dissertation, Cleveland State University, Cleveland, OH, 2013.

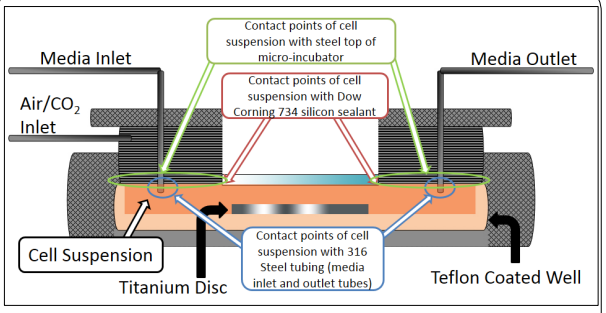
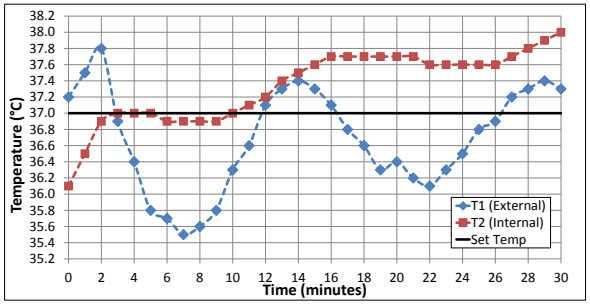


Figure 1. A side-view schematic of the micro-incubator system as though it were sliced in half. Highlighted are the contact areas between the cell suspension and the three "poisons" in the micro-incubator environment. Also noted are the main components of the micro-incubator system. **Note:** Image is not drawn to scale.

RESULTS AND DISCUSSION



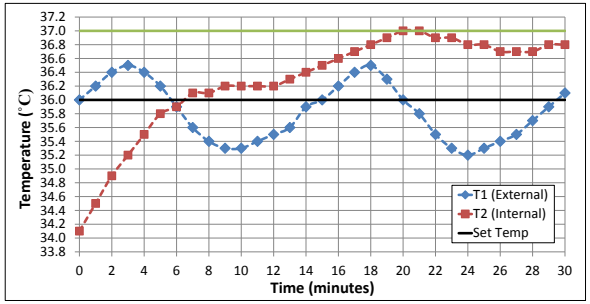
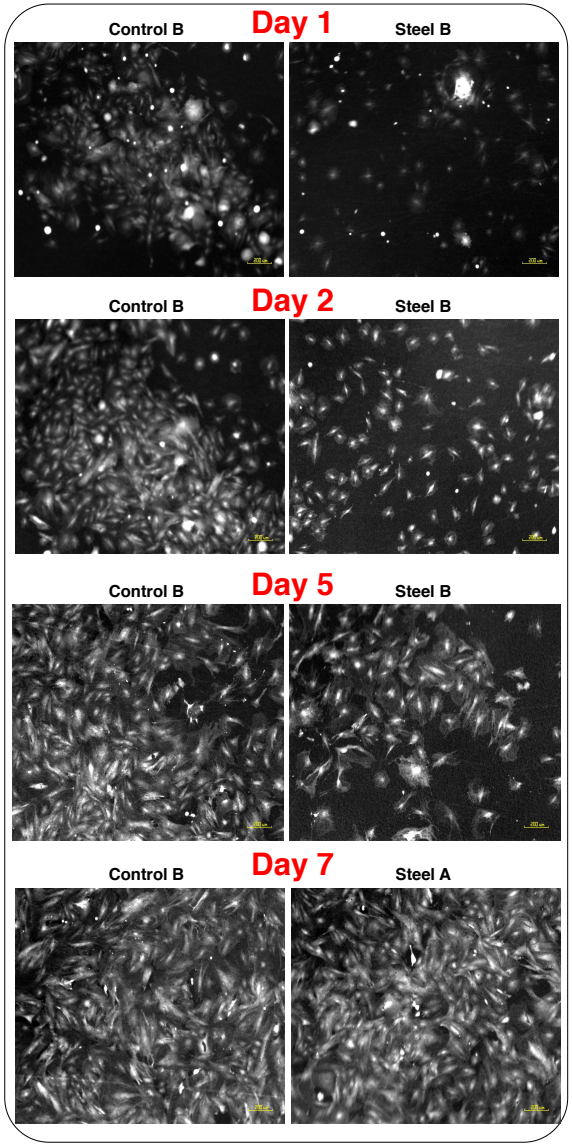


Figure 2. (TOP) Internal and external temperatures of the micro-incubator as a function of time for Channel 1 with the set temperature at 37°C. **(BOTTOM)** Internal and external temperatures of the micro-incubator as a function of time for Channel 1 with the set temperature at 36°C.

CONCLUSIONS

From the temperature experiments, it is clear that the internal temperature of the micro-incubator was higher than the intended set point temperature. It is possible that due to the continued exposure to temperatures near 38.0°C, the cells' proteins began to denature, causing the cells to function abnormally, and possibly leading to premature death. A set temperature of 36.2°C is thus recommended for all future experiments.

Based on a visual qualitative comparison of the control pictures versus those of the wells/discs with the steel and silicon poison present, there is little to no difference between the cell attachment and spreading. Thus, it can be concluded that the micro-incubator materials were not responsible for the premature cell death seen previously.



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